DATA EVALUATION RECORD

TRIFLUMEZOPYRIM

STUDY TYPE: 28- DAY RANGE FINDING -MOUSE

(NON-GUIDELINE)

MRID 49382232

Prepared for
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Task 6-169

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Template version 09/11

TXR#: 0057438

DATA EVALUATION RECORD[1]

STUDY TYPE: 28-Day Range Finding Dietary - Mouse
Non-Guideline.

PC CODE: 129210 **DP BARCODE:** D432127

TEST MATERIAL (PURITY): Triflumezopyrim technical (99% a.i.)

SYNONYMS: DPX RAB55; 2,4-Dioxo-1-(5-pyrimidinylmethyl)-3-(3-(trifluoromethyl)phenyl)-2H-pyrido(1,2-a)pyrimidinium inner salt

CITATION: Anand, S.S. (2015); DPX-RAB55 technical: Repeated-dose oral toxicity 28-day feeding study in mice. DuPont-33435. DuPont Haskell Laboratory, Newark, Delaware, USA. Testing Facility Report No.: DuPont-33435. Study date: May 5, 2015. MRID No. 49382232.

SPONSOR: E.I. du Pont de Nemours and Company

EXECUTIVE SUMMARY:

In a 28- day range-finding study in mice (MRID 49382232), male and female Crl:CD1(ICR) mice (10/sex/group) received triflumezopyrim in their diets at 0, 200, 800, 2500, or 7000 ppm (Limit Dose) for approximately 28-days (33 and 34 days for males and females, respectively). These were equivalent to 0, 34, 129, 416, and 1104 mg/kg bw/day in males and 0, 41, 161, 504, and 1343 mg/kg bw/day in females, respectively. Body weights, food consumption, and detailed clinical observations were evaluated weekly and acute clinical observations were evaluated daily. Hepatic biochemical parameters and clinical and anatomical pathology endpoints were evaluated at the end of the exposure period.

No treatment-related mortality, clinical signs, or effects on body weight or nutritional parameters were observed at any dose level. There were no adverse changes in clinical pathology parameters at any dose level. Slight decreases in red cell mass parameters were observed in male and female mice at 7000 ppm. These alterations were not considered adverse based on their minimal and transient nature. There was also an absence of such changes in studies of longer duration in mice (MRID 49382160 and 49382174).

There were no gross or histopathological changes in either sex at any dose level. Liver effects included dark discoloration (males, 7000 ppm), increased weights (males and females, ≥ 2500 ppm),

[1] This DER was generated by modifying the study summary in a Tier II document (MRID 49382105).

and hepatocellular hypertrophy (males, ≥ 2500 ppm; females, 7000 ppm). The liver effects were consistent with the pharmacological induction of hepatic enzymes and were interpreted to be non-adverse. Increased spleen weights were observed; however, there were no corroborating histopathological lesions observed. Accessory sex organ (ASO) weights were decreased in males fed 2500 and 7000 ppm of the test substance, but were not considered treatment-related due to lack of corroborative histopathological changes in the sex organs in this study. There were also no organ weight or histopathological changes seen in studies of longer duration in mice (MRID 49382160 and 49382174).

Changes in hepatic enzyme parameters were observed in male and female rats. Triflumezopyrim increased hepatic peroxisomal β-oxidation activity, a measure of potential peroxisome proliferation, in female mice at 7000 ppm. Total hepatic microsomal cytochrome P450 enzyme content and cytochrome P450 4A1/2/3 were increased at 2500 and 7000 ppm in male and females. Cytochrome P450 2B1/2 was increased at 7000 ppm in males. Cytochrome P450 1A2 was increased at 2500 and 7000 ppm in females. Cytochrome P450 2E1 was increased at 2500 and 7000 ppm in males and at 200, 800, 2500, and 7000 ppm in females. The increases in hepatic enzymes were consistent with the non-adverse, adaptive response of increased metabolism that resulted in increased liver weights and hepatocellular hypertrophy. In the absence of other effects indicative of liver injury (e.g., alterations in relevant clinical chemistry parameters and histopathology), changes in liver weights and hypertrophy were considered to be non-adverse, adaptive responses to exposure to a xenobiotic.

Under the conditions of this 28-day feeding study in mice, the No-Observed-Adverse-Effect Level (NOAEL) for male and female CD1 mice was 7000 ppm (1104 mg/kg bw/day and 1343 mg/kg bw/day, respectively), the highest dietary concentration tested, based on the absence of adverse changes in any of the parameters assessed. A Lowest-Observed-Adverse Effect-Level (LOAEL) was not established.

This 28-day oral toxicity study in mice is classified as **Acceptable /Non-guideline** since this is not a guideline requirement.

COMPLIANCE: This range-finding study was a non GLP study.

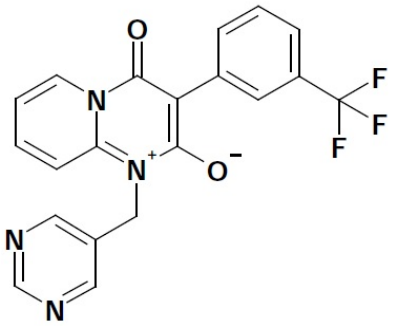
I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Triflumezopyrim technical

Lot/Batch #: RAB55-028
Purity: 99%
Description: Bright yellow solid
CAS # 1263133-33-0
Stability of test compound: Analyses confirmed that test material was stable in feed for at least 22 days at room temperature.

Structure:



2. Vehicle and/or negative control: Untreated diet

3. Test animals:

Species: Mouse
Strain: Crl:CD(SD)
Age at initial dosing: Approximately 50 days old
Weight at initial dosing: 27.0 -33.4 g for males; 21.2 – 26.4 g for females
Source: Males: Charles River Laboratories, Inc., Raleigh, NC.
Females: Charles River Laboratories, Inc., Kingston, NY.
Acclimation period: 7 days
Diet: PMI® Nutrition International, LLC Certified Rodent Lab Diet (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water: Tap water, *ad libitum*
Housing: Male mice were singly housed and female mice were pair-housed in solid-bottom caging with bedding mixed with enrichment.

4. Environmental conditions:

Temperature: 20–26°C
Humidity: 30–70%
Air changes: at least 10 air changes/hr
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. In-life dates: Start: August 10, 2011 - End: September 13, 2011

2. Animal assignment: Five groups of 10 animals/sex/dose level received triflumezopyrim

in their diets at concentrations 0, 200, 800, 4000, or 7000 ppm for 33 days. Males received 0, 34, 129, 416 and 1104 mg/kg bw/day, respectively, and females received 0, 41, 161, 504 and 1343 mg/kg bw/day, respectively. The 7000 ppm concentration was selected to produce a limit dose exposure (approximately 1000 mg/kg bw/day) and some toxicity, and the other concentrations were selected to assess a dose response for any observed effects and to establish a NOAEL. Animals were assigned to dose groups by computerized, stratified randomization so that there were no statistically significant differences among group body weight means within a sex. The negative control group (0 ppm) received untreated diet. The first 5 mice in each group were designated for repeated-dose toxicity evaluation, and the remaining 5 mice per group were designated for biochemical evaluation.

Table 1. Study Design: 28-Day feeding study in mice

Males				Females			
Group No.	**No. of Animals/group**	**Concentration in diet (ppm)[a]**	**Mean daily intakes (mg/kg bw/day)**	**Group No.**	**No. of Animals/ Group**	**Concentration in diet (ppm)[a]**	**Mean Daily Intakes (mg/kg bw/day)**
1	10	0 (control)	0 (control)	1	10	0 (control)	0 (control)
2	10	200	34	2	10	200	41
3	10	800	129	3	10	800	161
4	10	25,000	416	4	10	2500	504
5	10	7000[b]	1104	5	10	7000[b]	1343

[a] Weight/weight concentration of test substance

Note: The first 5 mice in each group were designated for repeated-dose toxicity evaluation, and the remaining 5 mice per group were designated for biochemical evaluation. However, mice designated for biochemical evaluation were included in the in-life data collection and anatomic pathology (except for liver pathology). Samples for hematology were collected from repeated-dose toxicity evaluation animals. Samples for clinical chemistry parameters were collected both from repeated-dose toxicity evaluation animals and from biochemical evaluation animals.

2. Diet Preparation and Analysis: The test substance was added to the rodent diet and thoroughly mixed to ensure homogenous distribution in the diet. Control diets were mixed for the same period of time. Diets were stored at room temperature until used. The homogeneity and concentration of triflumezopyrim in the dietary mixtures were checked by analysis using HPLC with ultraviolet (UV) detection at the beginning of the study. Concentration of triflumezopyrim in the dietary mixtures was also checked at the end of the study. The test substance was at target concentrations (± 7.7% of nominal), homogeneous (RSDs ≤3%) throughout the feed. Stability (22 days at room temperature) of test substance in feed to cover the concentration range of this study was verified in a concurrently conducted rat study. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

3. Statistics

Table 2. Statistical Analyses: 28-Day Feeding Study in Mice.

Parameter	Preliminary test	If preliminary test was not significant	If preliminary test was significant

Body weight Body weight gain Food consumption Food efficiency Clinical pathology[a,c] Organ weight	Levene's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance followed by Dunnett's test	Transforms of the data to achieve normality and variance homogeneity were used. The order of transforms attempted was log, square-root, and rank-order. If the log and square-root transforms failed, the rank-order was used.
Cytochrome P450 (total and isozymes) β-Oxidation UDPGT Hormone levels	Levene's test for homogeneity and Shapiro-Wilk test for normality[b]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.10, 0.05 was used for any calculations performed with those data. When an individual observation was recorded as being greater than a certain value, calculations were performed on the recorded value. For example, if specific gravity was reported as >1.083, 1.083 was used for any calculations performed with those data.

[b] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

[c] The number of samples analysed per clinical chemistry parameter varied across groups due to prioritisation of clinical chemistry parameters and insufficient sample volume. Statistical analysis was not performed on clinical chemistry parameters in which sample availability resulted in a group size of n = 1.

C. METHODS:

1. **Observations:** Animals were observed at least twice daily for mortality, morbidity and for signs of abnormal behavior and appearance. On days when they were weighed, each animal was individually handled, examined for abnormal behavior and appearance, and subjected to detailed clinical observations.

2. **Body Weights**: All animals were weighed once per week. Additional body weights were collected during the first week to track body weight gain.

3. Food consumption and compound intake: During the test period, the amount of food consumed by each mouse over the weekly weighing interval was determined by weighing the feeder at the beginning and end of the interval and subtracting the final weight from the initial weight divided by the number of rats in the cage. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. **Hematology and Clinical Chemistry:** Blood samples were collected approximately 5 weeks after initiation of the study. Samples for hematology were collected from repeated-dose toxicity evaluation animals and samples for clinical chemistry parameters were collected all animals. At sacrifice blood and bone marrow were collected. Blood samples for coagulation testing were not obtained. Hematology and clinical chemistry analyses were performed on the samples. Bone marrow smears were prepared but analysis was not necessary to support experimental findings.

   The following hematology parameters were determined: red blood cell count; red cell distribution width hemoglobin; absolute reticulocyte count hematocrit; platelet count; mean corpuscular volume; white blood cell count mean corpuscular; hemoglobin; differential white blood cell count mean corpuscular hemoglobin concentration;

prothrombin time and activated partial thromboplastin time.

The following clinical chemistry parameters were determined: aspartate aminotransferase; alanine aminotransferase; sorbitol dehydrogenase; alkaline phosphatase; total bilirubin; urea nitrogen; creatinine; cholesterol; triglycerides; glucose, total protein; albumin; calcium; globulin; inorganic phosphorus; sodium; potassium; chloride; and bile acids.

5. **Biochemistry/mechanistic parameters:** At sacrifice, the whole liver from each mouse designated for biochemical evaluation (5 mice/sex/group) was snap frozen in liquid nitrogen and stored between approximately -60°C and -80°C. Hepatic microsomes and peroxisomes were then prepared by differential centrifugation. The microsomal suspensions were analyzed for UDPGT activity, total cytochrome P450 content, and quantification of individual cytochrome P450 isozymes 1A1, 1A2, 2B1/2, 2E1, 3A2, and 4A1/2/3. The protein content of the microsomes was determined before analysis by the Biorad method.

6. **Sacrifice and Pathology:** At termination, animals were sacrificed by isoflurane anesthesia and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 3. Group mean organ weight values and organ weight ratios (% body weight and % brain weight) were calculated. Tissues collected from animals receiving the highest dose (7000 ppm) and control (0 ppm) were processed to slides and evaluated microscopically (Table 3). Gross lesions and suspected target tissues (male and female livers; male kidneys and spleens), as determined by examination of the control and high dose animals, were processed to slides and examined microscopically for all animals.

Table 3. 28-Day Feeding Study in Mice: Organs/tissues collected for pathological examination.

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Brain[a]	X	X
Spleen	X	X
Heart	X	X
Liver[b]	X	X
Kidneys[c]	X	X
Oesophagus		X
Adrenal glands[c]	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands[c, d]		X
Pancreas		X
Gall bladder		X
Skin		X

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Trachea		X
Nose (four levels)		X
Larynx/pharynx		X
Thymus	X	X
Mesenteric lymph node		X
Mandibular lymph node[c]		X
Bone marrow[e]		X
Peyer's patches[f]		X
Thyroid gland		X
Parathyroid glands[c]		X
Eyes (including retina and optic nerve)[c, g]		X
Testes[c, g]	X	X
Epididymides[c, g]	X	X
Prostate		X
Seminal vesicles (with coagulating glands)[c]		X
Ovaries (including oviducts)[c]	X	X
Uterus (including cervix)	X	X
Vagina		X
Mammary glands (females)		X
Accessory sex organs[h]	X	
Stomach		X
Pituitary gland		X
Lungs		X
Spinal cord[i]		X
Sciatic nerve		X
Optic nerves[c]		X
Skeletal muscle[j]		X
Femur/knee joint		X

[a] Brain includes cerebrum, cerebellum, midbrain, and medulla/pons.
[b] Microscopic evaluation of liver was conducted only from repeated-dose toxicity animals; livers collected from biochemical evaluation were snap frozen immediately after collection and used for enzyme assays.
[c] Both left and right organs.
[d] Salivary glands included mandibular, sublingual, and parotid glands.
[e] Bone marrow was collected with the femur and sternum.
[f] Peyer's patches were collected with the intestines.
[g] Fixed in Davidson's solution.
[h] Prostate + seminal vesicles + coagulating glands with their fluids
[i] Spinal cord includes cervical, mid-thoracic, and lumbar sections
[j] Biceps femoris skeletal muscle.
[k] Gross observations made at necropsy for which histopathology was not appropriate (*e.g.*, fluid, ruffled fur) were generally not collected.

II. RESULTS AND DISCUSSION:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were observed in either male or females at any dose level.

2. **Mortality:**
   No treatment-related mortality occurred in males or females at any dose level.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

There were no test substance-related effects on body weights or body weight gains.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY:

There were no treatment-related effects on food consumption or food efficiency.

D. CLINICAL PATHOLOGY:

1. **Hematology:** Lower group mean red cell mass parameters (red blood cell [RBC], hemoglobin [HGB] and hematocrit [HCT]) were observed in males and females fed 7000 ppm. Red cell mass parameters were 88–91% of controls (variable statistical significance) in males and 91-94% of control (not statistically significant) in females. In females, these changes were associated with a minimal increase (139% of control; statistically significant) in absolute reticulocyte count (ARET) suggesting a regenerative response to the minimal decreases in red cell mass. The changes in hematology parameters in the 7000 ppm male and female groups were minimal and transient nature, these changes are considered non-adverse. There were no other statistically significant or test substance-related hematology findings.

2. **Clinical chemistry:** No adverse, test substance-related effects on clinical chemistry parameters were observed. Cholesterol (CHOL) was higher in male and female mice dosed with 7000 ppm (161 and 155% of control, respectively; statistically significant). These values were considered to be likely spurious, as there were no associated changes in triglyceride levels and no differences in cholesterol levels in a 90-day study at similar concentrations, and they were considered to be non-adverse. There were no changes in triglycerides for either sex.

E. BIOCHEMISTRY/MECHANISTIC PARAMETERS:

Triflumezopyrim caused increases in the following liver biochemical parameters: peroxisomal β-oxidation activity (7000 ppm in females), total microsomal cytochrome P450 enzyme content (2500 and 7000 ppm male and females, cytochrome P450 1A2 (2500 and 7000 ppm in males and females), cytochrome P450 2B1/2 (7000 ppm males), cytochrome P450 2E1 (2500 and 7000 ppm males and 200, 800, 2500, and 7000 ppm females), and cytochrome P450 4A1/2/3 (2500 and 7000 ppm male and females). No

effects on UDPGT activity were attributed to test substance exposure in either sex. The effects on hepatic enzymes at dietary concentrations of 2500 and 7000 ppm triflumezopyrim were accompanied by statistically significant increases in relative (to final body weight) liver weight and liver hypertrophy. In the absence of clinical chemistry changes or histopathologic evidence of hepatic cellular injury, the changes noted in biochemical parameters were considered to be not adverse and were consistent with an adaptive response of increased metabolism due to exposure to xenobiotics.

Table 4. 28-Day feeding study in mice: biochemistry parameters (28-day sacrifice)

Parameter	0 ppm	200 ppm	800 ppm	2500 ppm	7000 ppm
Males					
Total P450 (nmol/mg protein)	0.511	0.461	0.647	0.740[a]	0.925[a]
UDP-GT (nmol/min-mg)	17.4	14.9	19.0	26.2	27.5
Cytochrome P450 1A1	375,071	375,129	372,484	384,415	374,016
Cytochrome P450 1A2	548,288	578,394	639,344	806,005[a]	1,023,965[a]
Cytochrome P450 2B1/2	485,710	500,759	505,348	469,983	679,911[a]
Cytochrome P450 2E1	682,570	666,713	681,912	799,347[a]	876,525[a]
Cytochrome P450 3A2	482,783	489,923	474,987	472,528	470,760
Cytochrome P450 4A1/2/3	415,204	451,458	457,849	494,154[a]	591,102[a]
β-oxidation rate (nmol/min-mg)	14.9	17.5	17.2	16.1	16.0
Females					
Total P450 (nmol/mg protein)	0.471	0.441	0.545	0.681[a]	0.878[a]
UDP-GT (nmol/min-mg)	14.0	10.1[a]	9.4[a]	12.1	17.6[a]
Cytochrome P450 1A1	322,936	326,950	328,483	332,089	330,161
Cytochrome P450 1A2	417,961	411,184	483,653	593,958[a]	729,211[a]
Cytochrome P450 2B1/2	538,149	557,565	529,189	550,637	585,699
Cytochrome P450 2E1	637,762	708,690[a]	711,379[a]	724,348[a]	753,554[a]
Cytochrome P450 3A2	447,425	501,385	447,592	394,924	410,197
Cytochrome P450 4A1/2/3	462,072	479,206	509,013	584,630[a]	621,596[a]
β-oxidation rate (nmol/min-mg)	7.9	7.5	8.2	10.3	13.6[a]

[a] Significantly different from control, $p < 0.05$.
Cytochrome P450 isozyme values units are intensity.

F. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** No adverse changes in mean organ weights were observed at any dietary concentration. As shown in Table 5, liver weights were increased in males and females at 2500 and 7000 ppm dose groups. The increases are consistent with the induction of hepatic metabolic enzymes and are considered to be non-adverse.

   As shown in Table 6, spleen weights were increased in males at 2500 and 7000 ppm groups and in females at 7000 ppm. There were no corroborating histopathological findings to support adversity of this change in organ weight.

   As shown in Table 7, accessory sex organ (ASO) weights were decreased in males fed 2500 and 7000 ppm of the test substance, but were interpreted to be non-adverse as there were no correlative microscopic changes and no effects on ASO weight or microscopic pathology in the 90-day mouse feeding study. All other individual and mean organ weight differences were considered to be spurious and unrelated to test substance administration.

Table 5. Mean Absolute and Relative Liver Weights in Male and Female Mice.

Group:	1	2	3	4	5
Dietary Concentration (ppm):	**0**	**200**	**800**	**2500**	**7000**
Male (number of mice)	(10)	(10)	(10)	(10)	(10)
mean final body wt. (grams)	35.5	34.4	35.0	34.8	35.8
absolute wt. (grams)	1.791	1.702	1.817	2.064 [b]	2.928 [a, b]
liver wt./brain wt. x 100	366.608	362.546	370.339	423.815 [b]	612.110 [a, b]
liver wt./body wt. x 100	5.034	4.967	5.183	5.912 #	8.144 [a, b]
Female (number of mice)	(10)	(10)	(10)	(10)	(10)
mean final body wt (grams)	30.0	28.9	28.5	30.2	29.4
absolute wt. (grams)	1.461	1.344	1.426	1.663 [b]	2.280 [a, b]
liver wt./brain wt. x 100	291.620	270.419	289.510	330.780 [b]	477.427 [a, b]
liver wt./body wt. x 100	4.832	4.637	4.995	5.495 [b]	7.737 [a, b]

[a] Statistically significant (Dunnett 2-sided p<0.05; parametric), compared to Group 1 (control).
[b] Values were interpreted to be test substance-related increases, compared to Group 1 (control).
[c] Statistically significant (Dunnett 2-sided p<0.05; non-parametric), compared to Group 1 (control).

Table 6. Mean Absolute and Relative Spleen Weights in Male and Female Mice

Group:	1	2	3	4	5
Dietary Concentration (ppm):	**0**	**200**	**800**	**2500**	**7000**
Male (number of mice)	(10)	(10)	(10)	(10)	(10)
mean final body wt. (grams)	35.5	34.4	35.0	34.8	35.8
absolute wt. (grams)	0.093	0.088	0.082	0.105 [b]	0.110 [b]
spleen wt./brain wt. x 100	19.079	18.801	16.672	21.414 [b]	22.935 [b]
spleen wt./body wt. x 100	0.261	0.258	0.234	0.299 [b]	0.307 [b]
Female (number of mice)	(10)	(10)	(10)	(10)	(10)
mean final body wt (grams)	30.0	28.9	28.5	30.2	29.4
absolute wt. (grams)	0.110	0.102	0.109	0.109	0.136 [a, b]
spleen wt./brain wt. x 100	21.961	20.637	21.913	21.633	28.281 [a, b]
spleen wt./body wt. x 100	0.363	0.354	0.383	0.360	0.460 [a, b]

[a] Statistically significant (Dunnett 2-sided p<0.05; parametric), compared to Group 1 (control).
[b] Values were interpreted to be test substance-related increases, compared to Group 1 (control).
[c] Statistically significant (Dunnett 2-sided p<0.05; non-parametric), compared to Group 1 (control).

Table 7. Mean Absolute and Relative Accessory Sex Organs Weights in Male Mice

Group:	1	2	3	4	5
Dietary Concentration (ppm):	**0**	**200**	**800**	**2500**	**7000**
Male (number of mice)	(10)	(10)	(10)	(10)	(10)
mean final body wt. (grams)	35.5	34.4	35.0	34.8	35.8
absolute wt. (grams)	0.401	0.386	0.410	0.348 [b]	0.321 #
ASO wt./brain wt. x 100	82.391	82.417	83.565	71.665 [b]	67.111 *
ASO wt./body wt. x 100	1.132	1.123	1.178	1.004 [b]	0.898 #

[a] Statistically significant (Dunnett 2-sided p<0.05; parametric), compared to Group 1 (control).
[b] Values were interpreted to be test substance-related increases, compared to Group 1 (control).
[c] Statistically significant (Dunnett 2-sided p<0.05; non-parametric), compared to Group 1 (control).

2. **Gross pathology:** Test substance-related gross morphological changes were observed in four of the 7000 ppm males and consisted of dark discoloration of the livers that correlated with hepatocellular hypertrophy observed at this concentration. All other gross observations were consistent with normal background lesions in mice of this age and strain.

3. **Histopathology:** An increased incidence of hepatocellular hypertrophy was observed in 7000 ppm males and females and in 2500 ppm males. This was attributed to a non-adverse, adaptive response to xenobiotic exposure (Table 8). In males at 2500 ppm, the hypertrophy was graded minimal (grade 1 of 4) in all affected mice; at 7000 ppm, it was graded moderate (grade 3 of 4) in all affected mice. In females, at 7000 ppm the hypertrophy was graded moderate in all affected mice. Microscopic hypertrophy was not observed at concentrations of 2500 ppm (females) or below.

   Increased splenic extramedullary hematopoiesis (EMH) was observed in 1/10, 2/10, 1/10, 3/10, and 4/10 mice in the 0, 200, 800, 2500, and 7000 ppm concentration groups, respectively. Severity scores were minimal to mild.

Table 6. 28-Day Feeding Study in Mice: Incidence hepatocellular hypertrophy.

Group:	**1**	**2**	**3**	**4**	**5**
Dietary Concentration (ppm):	**0**	**200**	**800**	**2500**	**7000**
Number of Animals/Sex	5	5	5	5	5
Males with hepatocell hypertrophy	0	0	0	**5**	**5**
Females with hepatocell hypertrophy	0	0	0	0	**5**

III. DISCUSSION AND CONCLUSION:

A. INVESTIGATORS CONCLUSION:

The NOAEL for males and females was 7000 ppm (1104 mg/kg bw/day and 1343 mg/kg bw/day, respectively), the highest dietary concentration tested, based on the absence of adverse changes in any of the parameters assessed.

B. REVIEWER COMMENTS:

The reviewer concurs with the investigators conclusion that the NOAEL is the highest dose tested. A LOAEL was not established.

C. DEFICIENCIES:

None